THE EFFECT OF ANESTHETICS ON THE SURFACE PRECIPITATION REACTION

L. V. HEILBRUNN

Department of Zoölogy, University of Pennsylvania

Living substance is unique in its capacity to respond to sudden changes in its environment. This innate irritability may, however, be lost temporarily under the influence of various anesthetics. In any general interpretation of irritability, the phenomenon of anesthesia must be carefully considered, and, indeed, if we could explain anesthesia we would be well on our way toward an interpretation of what actually happens when protoplasm is aroused to activity. It is not surprising, therefore, that so many authors have attempted to solve the riddle of anesthesia. Much of this work is summarized in Winterstein's excellent monograph (1926). A more recent review is that of Henderson (1930). The paper of Bancroft and Richter (1931) may also be consulted.

Some years ago, I began to gather data in favor of the view that stimulating agents cause a gelation of the protoplasm and that the primary action of anesthetics was to prevent such a gelation. This point of view was stressed in a monograph published in 1928. Facts were presented to show that stimulation does actually cause a very sharp increase in protoplasmic viscosity and that such general stimulating agents as the electric current or ultraviolet radiation do indeed stiffen the protoplasmic fluid. An attempt was also made to interpret the gelation involved in stimulation. Response was thought to be due to a type of clotting, the mechanism of which was essentially similar to that involved in the injury reaction which occurs when a cell is torn or broken. As the protoplasm begins to emerge from such a torn cell, a peculiar and distinctive type of precipitation reaction occurs. This I call the surface precipitation reaction, or s.p.r. A new film or membrane appears, and this membrane formation at the border of the exuding droplet may be accompanied by the formation of numerous new films within the droplet. As a result, many small vacuoles frequently appear, and this vacuolization may extend throughout the entire cell. Such a complete vacuolization usually leads to death, which is frequently the result of overstimulation. The gelation which occurs after normal stimulation is

thought to involve a reaction of the same general nature as the surface precipitation reaction, and it may be interpreted in terms of this reaction. In the present discussion, I shall limit myself to the simple concept outlined above, although the actual events may be much more complicated (compare Heilbrunn and Daugherty, 1933).

If, then, stimulation causes clotting of protoplasm, and if, moreover, this clotting is essentially an internal surface precipitation reaction, then it should follow, first, that anesthetics prevent clotting or gelation in the cell interior, and, second, that they should have an inhibiting effect on the s.p.r. In earlier work, I was able to show that ether and other fat solvents do actually exert a liquefying action on protoplasm, and do prevent the gelation which appears on stimulation (for details consult Heilbrunn, 1928). This much is in accord with theory. But, if the gelation which accompanies stimulation is fundamentally the same type of reaction as the s.p.r., then it should be possible to demonstrate an effect of fat solvents on the s.p.r. as it occurs when a cell is torn or broken.

Earlier attempts in this direction met with failure. When sea-urchin eggs were torn or broken in the presence of ether dissolved in anesthetic concentration in the surrounding sea water, the normal type of precipitation reaction occurred, and there was not the slightest evidence of any retarding effect of the ether. Various fat-solvent anesthetics were tried, and in no case was the s.p.r. retarded or prevented in their presence. Observations of this sort constituted a serious stumbling block to the point of view which I have been advocating.

It must be remembered, however, that when a sea-urchin egg is torn or broken in sea water, the surface precipitation reaction occurs in the presence of an abundance of free calcium. On the other hand, if an s.p.r. were to occur within the cell, the reaction would presumably take place in the presence of a very small amount of calcium, for there is good reason to believe that within the cell most of the calcium is bound chemically. In view of the fact that calcium plays such an essential rôle in the s.p.r., this is an important difference.

During the past summer, in the course of some experiments with the magnesium ion, I found that ether prevented the surface precipitation reaction which this ion may produce. It will be shown later that magnesium acts like calcium, except that its effect is much less potent. Thus, a dilute solution of calcium acts like a much stronger solution of magnesium. It was decided, therefore, to test the effect of ether on the s.p.r. in the presence of a very low concentration of the calcium ion. Under these conditions, it was indeed found that ether does prevent the s.p.r. Some of these observations will now be presented in detail.

Action of Ether on the S.P.R. in Arbacia

Only the simplest type of experiment was performed. In my original description of the s.p.r. in the *Arbacia* egg (Heilbrunn, 1927), it was shown that a trace of calcium was sufficient for the reaction. Thus if eggs are placed in isotonic sodium chloride solution and crushed, there is enough calcium carried over with the eggs to permit a reaction. If, however, eggs are washed once or twice in isotonic sodium chloride solution and then crushed, no reaction occurs.

Experiments were performed with solutions which contained 1000 parts of an isotonic sodium chloride solution (0.53 M) to one part of an isotonic calcium chloride solution (0.3 M). One drop of egg suspension was placed in 20 cc. of such a solution, and then after a minute a drop of the eggs was pipetted into a second dish also containing 20 cc. of the same solution. The eggs were then placed on a slide under a cover and crushed. The crushing was usually accomplished by sucking out fluid from the side of the cover slip with a piece of filter paper. When the pressure becomes great enough, the eggs break, the protoplasm streams out, and the small exuding droplet forms a membrane around itself. Pigment granules in the extraovate gradually disappear from view. There is thus a typical s.p.r. A peculiar phenomenon then follows in most of the eggs which are crushed in these solutions containing little calcium. Membrane elevation similar to that produced by the sperm in the fertilization reaction now occurs in that part of the egg which has remained intact. Starting from the rim of the extraovate, that is to say, from its junction with the uninjured protoplasm, the vitelline membrane slowly starts to lift away from the cytoplasm of the egg. This membrane elevation proceeds around the egg surface until the entire uninjured portion of the egg has a perfect fertilization membrane. It is believed that this observation of membrane elevation following injury is of real importance for the theory of membrane elevation, but I shall not discuss it further in this paper, except to point out that the phenomenon occurs only when the calcium concentration is low. In sea water there is no evidence of it.

If eggs are immersed as before in 1000 parts by volume of isotonic sodium chloride solution plus 1 part of isotonic calcium chloride solution, with now the addition of 2 or 3 cc. of ether per 100 cc. of solution, a different result is obtained. To prevent the evaporation of ether, the eggs are placed in glass-stoppered weighing bottles, but otherwise the treatment is the same. When the etherized eggs are crushed under a cover slip, no surface precipitation reaction occurs, and there is no subsequent membrane elevation. Usually the pigment granules emerging

from the crushed eggs remain intact and visible. The ether has thus interfered with the surface precipitation reaction, and has prevented both breakdown of pigment granules and formation of a new film or precipitation membrane. (Membrane elevation is also inhibited.)

Ether can thus prevent the s.p.r. in the *Arbacia* egg, but this antagonism occurs only when the calcium concentration is low. If the calcium concentration is doubled, that is to say, if the eggs are crushed in 500 parts of isotonic sodium chloride solution plus 1 part of isotonic calcium chloride solution, the effect of the ether is not so noticeable, and with slightly higher concentration of calcium, it disappears completely.

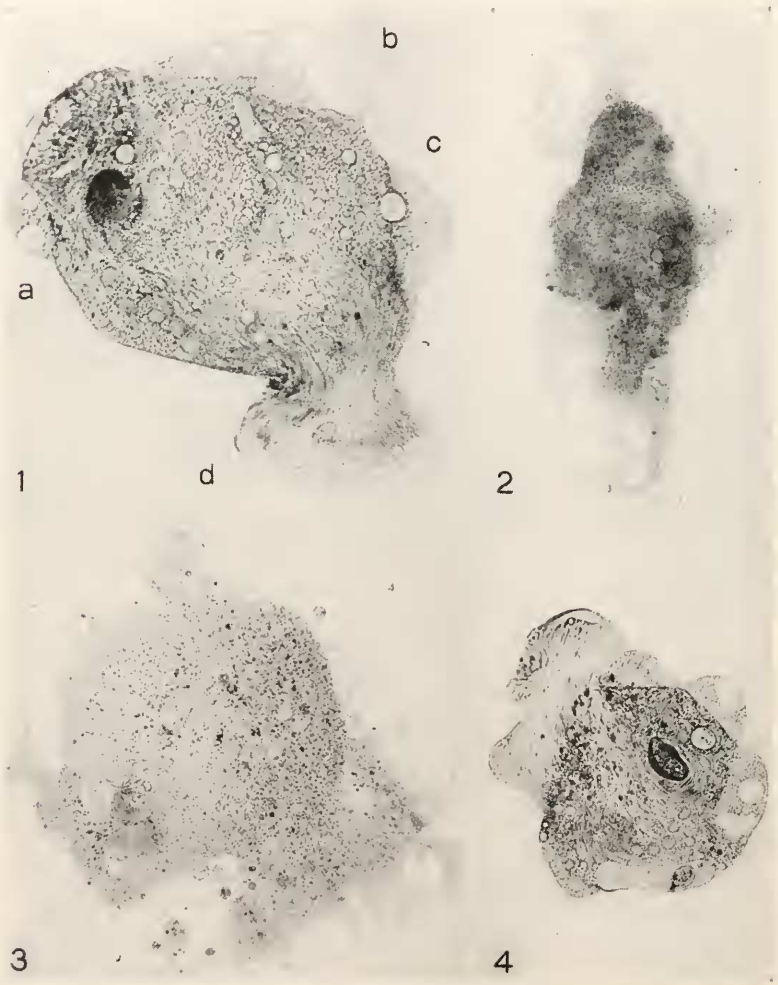
The action of ether on the s.p.r. may be demonstrated very simply by comparing the reaction in an isotonic sodium chloride solution with that which occurs in a similar solution containing 2 per cent ether (by volume). When a drop of egg suspension is added to 20 cc. of an isotonic sodium chloride solution, and the eggs are crushed, there is a clear-cut reaction involving both breakdown of pigment granules and formation of a new surface film. On the other hand, in the presence of ether, the reaction is inhibited. In performing this experiment, eggs should be tested within a few minutes after immersion into the solutions, for with increasing length of exposure to isotonic sodium chloride, the surface precipitation reaction becomes less distinct and the pigment granules tend to remain intact on escaping into the sodium chloride solution. A similar time effect can also be noted in the case of magnesium solutions.

Action of Ether on the S.P.R. in Stentor

The action of ether in suppressing the s.p.r. may be very clearly demonstrated for the protozoön *Stentor*. This form is ideal for a study of the reaction and when the cell membrane of the stentor is broken, a beautiful s.p.r. occurs. Through the kindness of Dr. Hetherington, I was able to secure an excellent culture of *Stentor cæruleus,* and numerous experiments were performed with it. Later other cultures were obtained in Philadelphia. In these the animals were reared in a dilute wheat infusion in pond water. Similar results were obtained with them.

When *Stentor* is placed on a slide, and a cover slip is pressed down over it so that the protoplasm is forced out through a rupture of the body wall, the emerging droplet immediately forms a membrane about itself. I know of no protozoön which gives a clearer reaction. Figure 1 is an unretouched photograph which shows the phenomenon clearly. Several droplets with their membranes appear at *a, b, c*. There is evi-

dently a sharp border between the protoplasm which has been pressed out of the cell and the surrounding medium. At *d* the protoplasm is moving out of the cell, and a membrane is just being formed. The s.p.r. in *Stentor* is also shown in Fig. 4, in which the background has been blocked out for purposes of contrast.



EXPLANATION OF PLATE I

FIGS. 1 AND 4. Surface precipitation reaction in *Stentor*.

FIGS. 2 AND 3. Absence of surface precipitation reaction in the presence of 1 per cent ether.

The magnification is × 80 for Figs. 1 and 3, and × 50 for Figs. 2 and 4.

If, now, stentors are immersed in 1 per cent ether in tap water (Woods Hole or Philadelphia) and crushed in the same manner as before, no s.p.r. occurs, and the protoplasmic granules stream through the solution. This is clearly shown in Figs. 2 and 3, unretouched photographs. In order to make certain that the effect is due to the ether and not to the tap water, control experiments were performed in which animals were crushed in tap water alone. In such tests, a perfect s.p.r. was obtained. It is evident, therefore, that in *Stentor,* as well as in *Arbacia,* ether exerts an inhibiting effect on the s.p.r. A concentration of ½ per cent ether is not as effective as 1 per cent ether in stopping the reaction. In 2 per cent ether, no typical s.p.r. occurs, but the protoplasmic granules from crushed specimens do not scatter through the solution as is the case when 1 per cent ether is used.

Results similar to those obtained with ether were noted when *Stentor* was immersed in 1 per cent butyl alcohol and crushed. Ethyl urethane, amyl alcohol, and acetone were also effective in preventing the s.p.r.

The results obtained indicate that ether (and other fat-solvent anesthetics) can exert an inhibiting effect on the s.p.r. This effect is more readily observed in *Stentor,* for it may be noted when ether is added to the solutions in which *Stentor* lives. On the other hand, in the case of sea-urchin eggs, in order to show the effect of ether, one must study its action in solutions which contain only a very small amount of calcium.

Moreover, in the case of sea-urchin eggs, when ether in relatively high concentration (3–4 per cent) is added to the sea water surrounding them, the eggs undergo a vacuolization reaction which is thought to be essentially an internal surface precipitation reaction. Thus, although under some conditions ether may inhibit the s.p.r., under other conditions it may actually initiate the reaction. The latter effect is believed to be due to the fact that ether releases free calcium into the egg interior. This free calcium then initiates the reaction even though ether is present.

It thus appears that ether (and presumably fat solvents generally) can both favor an s.p.r. and prevent it. We thus have a formal explanation of the queer fact that fat-solvent anesthetics are sometimes stimulants. In the case of the *Arbacia* egg, the gelating or stimulating effect of ether preponderates at higher concentrations of the fat solvent.

Relation of the Magnesium Ion to the S.P.R.

Most theories of anesthesia find the fact of magnesium anesthesia a stumbling block. They have no way of explaining it. Magnesium, it is true, may lower permeability, but its chief antagonist is calcium, which has a similar effect on permeability. This difficulty has bothered some of the adherents of the permeability doctrine, so much so that

Höber (1926) has been led to state that magnesium anesthesia is not a true anesthesia; for, says he, true anesthetics are all surface-active, they penetrate cells and paralyze all their functions reversibly, at least more or less, whereas magnesium acts primarily at cell junctions or synapses. In view of the fact that magnesium salts are perhaps the most widely used anesthetics for marine invertebrates, and have an anesthetic action on widely divergent organisms from amœba (Heilbrunn, 1932) to man, it is not so easy to dismiss them as anesthetics. Perhaps, after all, it is a question of definition. Personally, I doubt if magnesium is less of an anesthetic than ether.

How then shall we explain the action of the magnesium ion? In a recent note (Heilbrunn, 1932; see also Heilbrunn and Daugherty, 1932) it was shown that magnesium tends to liquefy the plasmagel of the amœba. This fact fits in well enough with the general theory of anesthesia outlined above, and it is also in accord with other evidence as yet unpublished which indicates that various types of anesthetics all liquefy the plasmagel of *Amœba proteus*. But there is another side to our theory, for stimulation is thought to involve a reaction related to the s.p.r. What effect then does the magnesium ion have on the s.p.r. and is it possible to relate such an effect to the known facts of anesthesia?

In my 1927 paper, the relation of magnesium to the s.p.r. was considered briefly and not very correctly. It was stated that magnesium was not a good substitute for calcium, and that when an s.p.r. occurred in magnesium solutions, it was due to a secondary process. As a matter of fact, magnesium may be substituted for calcium. Indeed, an excellent s.p.r. occurs in isotonic solutions of magnesium salts.

When *Arbacia* eggs are immersed in a solution of 0.3 M magnesium chloride, and are then crushed after a minute or two, there is a clear-cut reaction at the surface of the exuding droplet of protoplasmic fluid and an excellent membrane is formed. Moreover, the pigment granules break down, or at least some of them do. As a matter of fact, the behavior of the pigment granules in these eggs is very interesting. Typically, when a droplet of protoplasmic fluid begins to emerge from the torn surface of the egg, the pigment granules are at first intact in the exuded mass. Breakdown of granules then begins in a very limited region. The broken egg is dumbbell-shaped, consisting of a large sphere which is the original egg cell, and a smaller sphere, the exuded droplet or extraovate. Between the two there is a relatively narrow neck of material. It is in this neck, the original cortex of the egg in the injured area, that pigment-granule breakdown occurs. From this initial point of pigment-granule breakdown, a wave of reaction spreads in both directions into and across the original egg cell on the one side, and out into

the exuded droplet on the other. The phenomenon is very striking. As the pigment granules break down at any one point, they release a bright red color, and vacuoles come into view. At the next instant, neighboring granules go through the same process and the reaction is repeated across the cell. Often the wave of reaction goes completely across the original cell and the extraovate, so that all parts of the dumbbell are affected. The speed of the wave can readily be measured. Usually at room temperatures it takes approximately 15 seconds for the wave to pass to the farthest end of the cell. There is more or less variation in speed with different degrees of injury. Even so, fairly consistent values may be obtained. Thus, six tests showed 15, 14, 18, 14, 21, 15, 18 seconds. When eggs are crushed in isotonic calcium chloride solution, there is a similar wave of reaction across the cell. In this case, however, it appears to be more rapid, and the egg is usually traversed in about 5 seconds.[1] In calcium chloride solutions, breakdown cannot be seen to begin at the neck of the dumbbell, but occurs almost instantly in all of the exuded droplet. In solutions of magnesium chloride, the wave of reaction is often halted before it passes across the egg or before it crosses the extraovate. Thus, in eggs crushed in magnesium chloride solutions, one often sees intact pigment granules in the extraovate. Once the reaction wave has been halted, the pigment granules beyond its path seem to acquire a resistance to breakdown. This applies both to the granules within the egg proper and those in the extraovate.

When the s.p.r. is observed in sea water, the pigment granules in the extraovate all break down and there is also a wave of breakdown which passes across a fraction of the original egg. Usually the wave across the egg itself is halted before it proceeds very far.[2] If, now, the egg be crushed a second time, those pigment granules which resisted breakdown after the first injury lose their pigment and disappear. Eggs in magnesium chloride solutions behave differently. If they are crushed once, and if the wave of pigment-granule breakdown fails to include the whole egg, those granules beyond the path of the wave become resistant to breakdown. When the egg in magnesium solution is crushed a second time, the intact pigment granules do not break down, even though they float free in the surrounding medium. It is thus apparent that under the conditions described above, the pigment granules have acquired an immunity to breakdown.

It is possible that in this observation lies a clue to the nature of the anesthetic action of the magnesium ion. We can conceive of living

[1] These values for speed of reaction wave across the cell are for eggs crushed within a few minutes after immersion into the solutions. Following longer times of immersion, the waves move more slowly.

[2] This stoppage of the wave is apparently due to the presence of sodium ion.

protoplasm as being always in a state of unstable equilibrium between those factors which favor an s.p.r. and those which inhibit or reverse it. Then, once an s.p.r. occurs, it must, to some extent at least, be reversed before the protoplasm can become irritable again, so that normal stimulation in itself involves as a necessary sequence the reversal of the reaction involved in stimulation. It is possible that anesthetics sometimes act by causing a mild stimulation followed by an immediate reversal. The stimulation may be completely hidden so that the observer sees only the reversal, and there is apparently only anesthesia. The fact that in magnesium solutions granule breakdown, which may be regarded as a primary part of the stimulatory gelation, may be inhibited, opens the possibility of explaining the action of magnesium salts in terms of the theories outlined above. It is at least clear that under certain conditions, magnesium may be regarded as tending to inhibit the s.p.r.

However, this is not the only way in which the anesthetic action of magnesium may be interpreted in terms of the s.p.r. It has already been noted that both calcium and magnesium can initiate the s.p.r.[3] Calcium is effective in extremely low concentration. In experiments cited above, the s.p.r. was found to occur in solutions in which one part of isotonic calcium chloride solution was mixed with a thousand parts of isotonic sodium chloride solution. Actually, one part of calcium solution in two thousand parts of sodium solution is sufficient. Not so with magnesium; it must be present in much higher concentration. Experiments were performed in which eggs were placed in various mixtures of isotonic sodium chloride (0.53 M) and isotonic magnesium chloride (0.3 M). In such experiments it is essential to wash the eggs in one dish containing a given mixture and then transfer them to a second. In each transfer a single drop of egg suspension was carried over to 20 cc. of the solution. This procedure is necessary in order to wash the eggs relatively free from calcium ion. Apparently there is some variation between different lots of eggs, nor is it always easy to state precisely just when an s.p.r. occurs and when it is absent, for there is, of course, a gradual transition between a complete reaction and none at all. It is certain, however, that 1 part of magnesium solution to 39 parts of sodium solution is not sufficient to cause an s.p.r. when the eggs are crushed in this mixture. Neither is 1 part magnesium solution to 19 parts sodium solution. In mixtures of 1 part magnesium solution to 9 parts sodium solution, the reaction may or may not occur. This is a boundary concentration and mixtures which contain appreciably more than 1 part of isotonic magnesium solution in 10 parts of the total solu-

[3] It is possible that only calcium is directly involved in the reaction and that magnesium acts by releasing calcium from some chemical union in the protoplasm.

tion are certain to give the reaction. Thus, the magnesium ion is relatively weak in comparison with the calcium ion; the latter is at least a hundred times more powerful in promoting the s.p.r.

These observations suggest a second type of explanation for the anesthetic action of the magnesium ion. It is reasonable to assume that in living systems the magnesium ion can replace the calcium ion. It has been assumed (Heilbrunn, 1928; Heilbrunn and Daugherty, 1933) that the stimulating gelation within cells is due to a release of calcium ion. Suppose now that a cell anesthetized with magnesium has its calcium compounds to some extent replaced with magnesium. If the replacement were complete, stimulation would, according to our theory, require the release of at least 100 times as much free ion if this were magnesium instead of calcium. Thus the magnesium-treated cell becomes relatively resistant to stimulation.

General Discussion

When a living cell is aroused to activity, changes in its protoplasm must occur. These changes are almost certainly not confined to the osmotic membrane of the cell, and the main mass of the living substance is doubtless affected. Protoplasm is a colloidal material and we are therefore faced with a problem in colloid chemistry.

In interpreting stimulation and the action of anesthetics which prevent it, many authors have reasoned from the known effects of stimulating agents and anesthetics on inanimate colloids. The primary information concerning the colloid chemistry of protoplasm has clearly shown the inadequacy of such reasoning. Protoplasm is vastly different from any known inanimate colloid. It is, therefore, essential to study the effects of stimulants and anesthetics on protoplasm itself. Unfortunately, many of the cells which give the most interesting responses when stimulated can not readily be studied from a colloid chemical standpoint. If we are to make progress in this field, the only sure method at our disposal at the present time is to study the effects of stimulants and anesthetics on those cells whose protoplasm lends itself to colloid chemical study. Thus we can determine the effect of ether on various types of protoplasm, and the knowledge so gained is of more importance to the theory of anesthesia than any information derived from non-living materials.

All protoplasm is very sensitive to physical treatment and chemical reagents. It is easily injured, and any excessive stimulation leads to injury or death. Indeed, it is probable that stimulation is equivalent to slight injury. Almost all agents which stimulate or injure protoplasm cause a peculiar reaction in it. The fluid protoplasm becomes stiffer

and this gelation when carried to the point of injury is seen to involve a characteristic vacuolization reaction. Ultraviolet radiation, X-rays, the electric current, sudden pressure, heat, all produce this reaction. How shall we interpret it?

In earlier studies, I have shown that the vacuolization reaction is the same reaction as that which occurs when a cell is torn or broken, that is to say, it is essentially an s.p.r. In many cells the s.p.r. is very easy to study, and one can readily determine the effect of various reagents upon it. But it should be clearly understood that the s.p.r. is an extraordinarily complex reaction or series of reactions. In many respects it resembles blood coagulation. Probably the fundamental reaction of living cells, their power to clot when torn or broken, has been taken over by the blood of many animals, so that there is a deep underlying relationship. It may be remembered that in blood clotting, cell or tissue extracts play important rôles.

In spite of the numerous studies of blood clotting, the process is still very incompletely understood. Only very few authors have studied the clotting of the protoplasmic fluid, so that our knowledge in this field is only at the beginning.

If the attempt is made to develop a logical theory of stimulation based on our present knowledge of the colloidal chemistry of protoplasm, it should be possible in such a theory to account for the action of anesthetics. Apparently protoplasm is in a delicate state of balance between the forces which favor clotting or gelation and those which inhibit or prevent it. With minor reservations, it may be said that stimulation implies a gelation of the main mass of the protoplasm, and anesthesia a solation. If the gelation produced by stimulating agents is essentially an s.p.r., then it should be possible to show that anesthetics prevent or retard the s.p.r. That has been the purpose of the present contribution. For the first time, I have been able to show that fat-solvent anesthetics do actually prevent the s.p.r. in certain types of living material. Moreover, the relation of the magnesium ion to the s.p.r. indicates two possible ways in which this anesthetic ion may retard or prevent the reaction. It has thus been possible to add an additional step in the colloid chemical interpretation of stimulation and anesthesia.

Summary

1. In the presence of low concentrations of calcium, ether inhibits the surface precipitation reaction in *Arbacia* egg cells.

2. Ether and other fat solvents also inhibit the surface precipitation reaction in *Stentor* protoplasm.

3. The magnesium ion can cause a surface precipitation reaction in *Arbacia* eggs, but it is far less potent than calcium. The course of the reaction in magnesium solutions is described.

4. There are two ways in which the presence of magnesium might act to prevent the surface precipitation reaction.

5. The relation of these facts to the colloid chemical interpretation of anesthesia is discussed.

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